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Comparative Morphology of Ligules of Three Indian Species of *Selaginella*

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ABSTRACT.—The ligule morphology in three Indian species of the genus *Selaginella*, e.g., *S. bryopteris*, *S. repanda* and *S. panchghaniana* is described for the first time. Though the leaves (ventral and dorsal) in all the three species are typically normal, some dorsal leaves in *S. panchghaniana* have two ligules, bifid tips and lobed bases. The form of ligules is variable within each species but the overall shape (outline) is constant. The size of ligules may be influenced by environmental conditions. The presence of glandular cells at the margin of the tip of ligules indicates a water secreting function.

KEY WORDS.—*Selaginella*, leaves, ligule, lobed, shape and glandular cells

The ligule is a membranous tongue-like structure present on the adaxial surface of the leaf at the junction of leaf and stem. It is the characteristic feature of a number of extinct arborescent lycopsids, the Devonian herbaceous *Leclercqia* and extant genera such as *Sellaginella* and *Isoetes*. Bower (1959) realizing the importance of this small structure divided Lycopsida into the two groups Eligulatae and Ligulatae. Hofmeister (1851) was the first botanist to report the presence of ligules in the genus *Selaginella*. Since these reports, a number of workers (McNab, 1887; Campbell, 1895; Velenovsky, 1905; Verdoorn, 1938; Von Goebel, 1930) have given reference to or brief mention of ligules in their description of the genus.

Harvey Gibson (1896) first gave a detailed account of the structure, development and function of ligules of *Selaginella*. Ball (1925) reported the presence of double ligules within a single sporophyll in *S. martensii* Spring. Vink (1953) described the lobed ligules in *S. caulescens* (Wall.) Spring. Hsu (1937) and Lafont and Lemoigne (1965) gave the brief descriptions of the ligules of *S. sinensis* (Desv.) Spring and *S. willdenovii* (Desv.) Baker, respectively. Dahlen (1988) made a detailed observation of the ligules both on stems and branches of 11 species of *Selaginella* from Hong Kong and concluded that ligules of mature lateral leaves on branches are the least variable and most representative of a specimen. He also concluded that despite some variability, ligule shape may be independent of other morphological features and/or environmental influences and that it may be used to distinguish species.

Webster (1970) successfully developed aberrant leaves on the angle-shoot meristem in *S. martensii*. Kohlenback and Geier (1970) studied the function of

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ligules of *S. kraussiana* A. Braun and reported that the ligules were found to absorb Berberine sulfate in the apical region where the cuticle is thin. Bilderback (1987) found the association of mucilage in the ligules of a number of species of *Selaginella*.

Horner and Beltz (1975) described the development and fine structures of ligules in *S. pilifera* A. Braun and *S. uncinata* (Desv.) Spring. Lerbs (1974), Sigee (1974, 1975, 1976) and Bilderback and Slone (1987) described the ultrastructural and physiological details of ligules in *S. martensii* and *S. kraussiana* respectively. Jagels and Garner (1979) observed the variation in callose deposition in the ligules of *Selaginella*.

Shaw and Hickey (2005) have given a comparative account of *Selaginella* and *Isoetes*. The basic organization of the ligules of *Selaginella* and *Isoetes* is same, however the ligule of *Selaginella* is much simpler than that of *Isoetes* and lack elaborate glassopodium.

The survey of literature on *Selaginella* has revealed that very little attention has been paid to the studies of the ligules. As a result, this structure remains neglected especially by the Indian workers (Dixit, 1992) and others. No significant advancement has been made in the knowledge of *Selaginella* ligules since 1987, with the exception of a review by Pant *et al.* (2000) and a brief mention by Taylor *et al.* (2009). The present study was designed to increase our knowledge of ligule structure in *Selaginella*.

Material and Method

The specimens of different species of *Selaginella* Beauv. were collected from a number of localities in India: *S. bryopteris* (L.) Baker from Bhilaigarh (CH), Jargodam, Ahrora and Sidhnath fall (U.P.); *S. repanda* (Desv. ex Poir), from Matkuli and Apsravihar (M.P.); and *S. panchghaniana* Dixit, from Khandala, Panchgani Table-land and Bhose (MH). Habitat and the morphometric details were observed in the field. Fresh materials were fixed in FAA for further examination in the laboratory. Ten herbarium vouchers were prepared from each locality of each species and the specimens were deposited in Duthie Herberium, Department of Botany, University of Allahabad, Allahabad. The terminology followed in the paper is adopted from Shaw and Hickey (2005).

For the present investigation, the ligules of five plants per population and five leaves from each specimen were randomly selected for the studies of apical, middle and basal regions of both ventral and dorsal leaves. The ligules were dissected out from basal region of the leaf and mounted in Glycerin jelly. The size (length and breadth) of ligules were measured under light microscope and photographed with a Leica DMLB DC300 microphotographic microscope.

Results

Selaginella bryopteris is a non-rhizophoric species, the other two, *S. repanda* and *S. panchghaniana*, are rhizophoric species.

Ligules are present on both ventral and dorsal leaves of all of the three species of *Selaginella* (Fig. 1). In all three species both ventral and dorsal leaves are of the normal type, i.e., single leaf with single ligule, single tip and single base. However, sometimes in *S. panchghaniana* the dorsal leaves possess two ligules, bifid tips and two bases (i.e., single leaf with two ligules) (Fig. 1F, G). Both ligules are nearly the same size (Fig. 1F), but occasionally one of them is larger and than other (Fig. 1G).

In *S. bryopteris* the ligules are situated in a shallow cavity at the base of leaves with less marked swollen regions, but in *S. repanda* and *S. panchghaniana* the ligules appears just above the surface of leaves at their base. The position of ligules is parallel to mid-vein of leaves in *S. bryopteris* and *S. repanda* but oblique in *S. panchghaniana*.

The shape of ligules is variable. It is cup-shaped or somewhat fan-shaped in *S. bryopteris* (Fig. 2A–D), but horn-shaped or tongue-like in *S. repanda* (Fig. 2E–I) and only tongue-like in *S. panchghaniana* (Fig. 2J–L; Fig. 1A–C). Though the shape (outline) of the ligule is constant within species, minor morphological variation has been recorded among the ligules of the same specimen. Each ligule is composed of two major parts, glassopodium and tongue. The tongue consists of two sections, namely the bulbous base and the tip or neck. The glassopodium is represented by a few cells present at the base of the ligule. The cells of the bulbous base are hexagonal and dark in color in comparison to that of the tip or neck. The cells of tip or neck section are more elongated. The tip of ligule of *S. bryopteris* is multi- to tri- lobed and the margin of the tip is crenate (Fig. 2A–D), and those of *S. repanda* are tri-, bi-, or unlobed and the margin of the tip is obtuse (Fig. 2E–I). The ligules of *S. panchghaniana* are unlobed to vary rarely bilobed. The margin of the tips of ligules varies from obtuse to acute (Fig. 2 J–L; Fig. 1 A–C).

The size (length and breadth) of ligules varies among the species. It is 285–105 μm (L) × 457.5–82.5 μm (B) in *S. bryopteris*; 285–142.5 μm (L) × 135–67.5 μm (B) in *S. repanda* and 345–105 μm (L) × 105–37.5 μm (B) in *S. panchghaniana* (see Table 1).

Discussion

Harvey Gibson (1896) provided a detailed description of the structure, development and function of ligules in 50 species of *Selaginella*. He had remarked that "considerable variation occurs in the precise form of the ligule, even in the same plant; but, on the whole, within certain limits, the outline is maintained fairly constantly for the species". Our studies of *Selaginella* support the observations of Harvey Gibson (1896) and Dahlen (1988). The ligule shape of different species of *Selaginella* is characteristic and likely to be helpful in taxonomic delineation.

Normally each ventral and dorsal leaf is provided with one tip, one base and one ligule (Fig. 1D, E). However, we have observed that in some dorsal leaves of *S. panchghaniana* bifid tips, two inseparable basal lobes and two ligules of equal or unequal size (Fig. 1 F, G), are present. Previously, Ball (1925) noticed

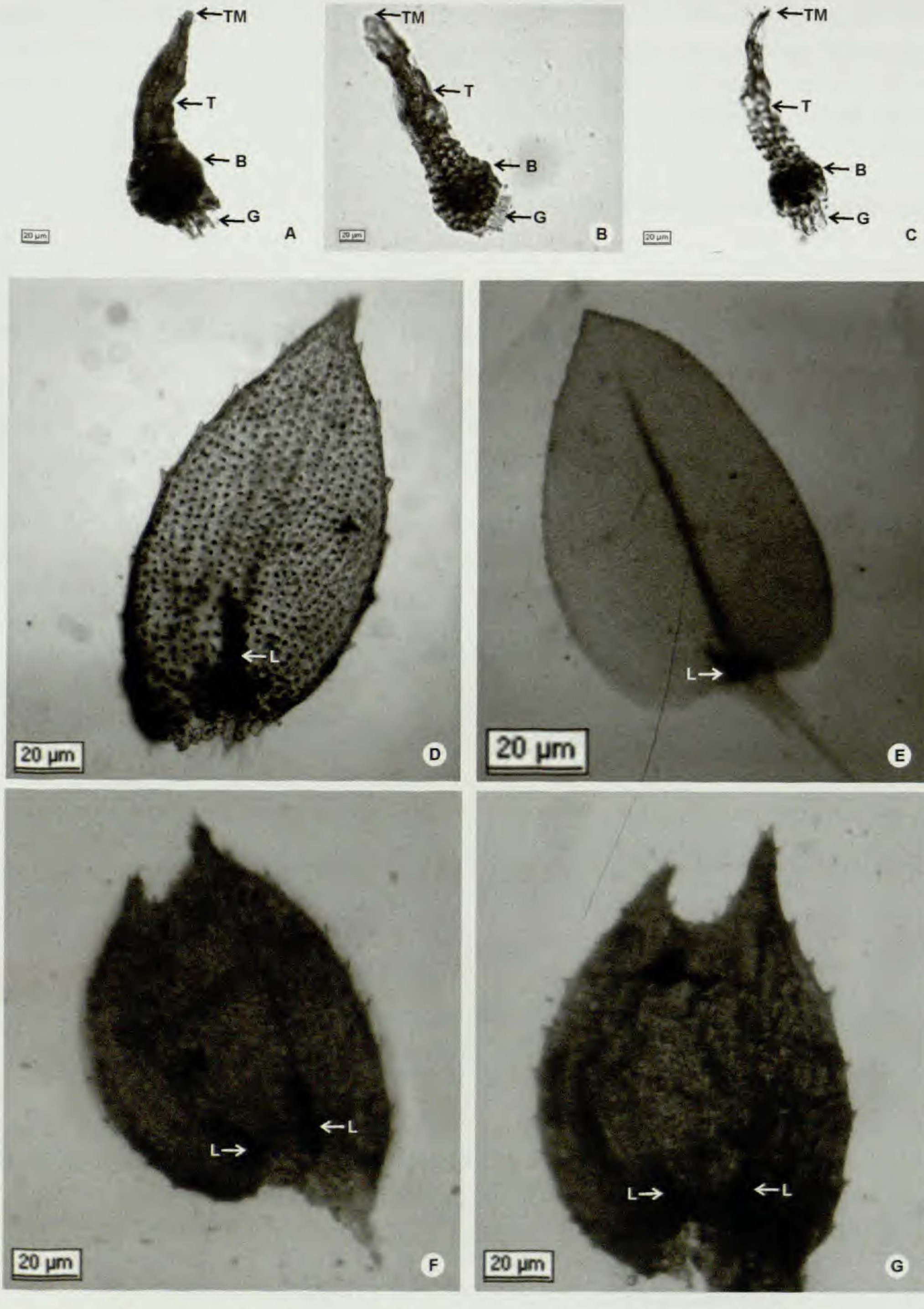
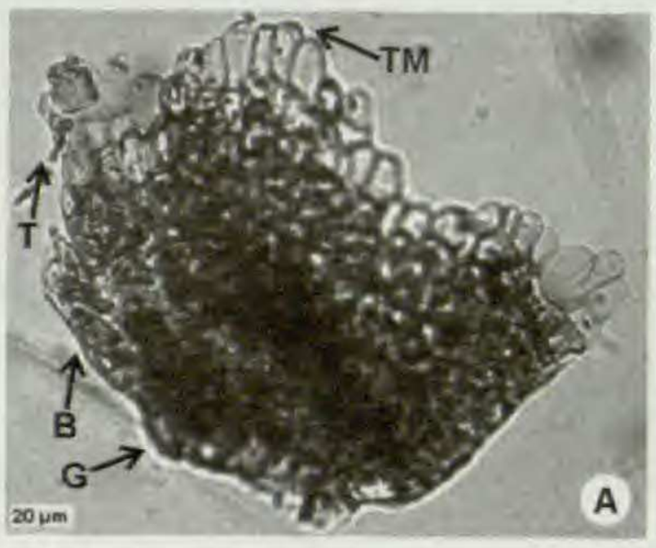
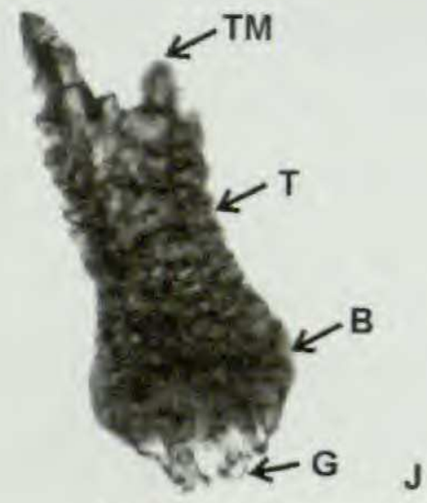
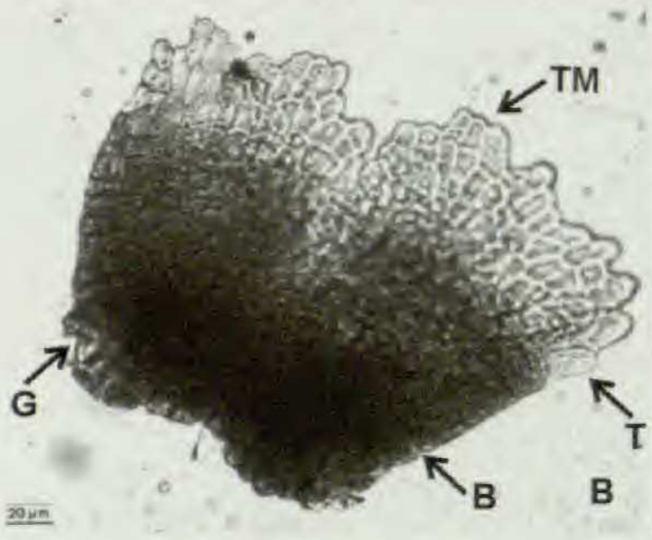
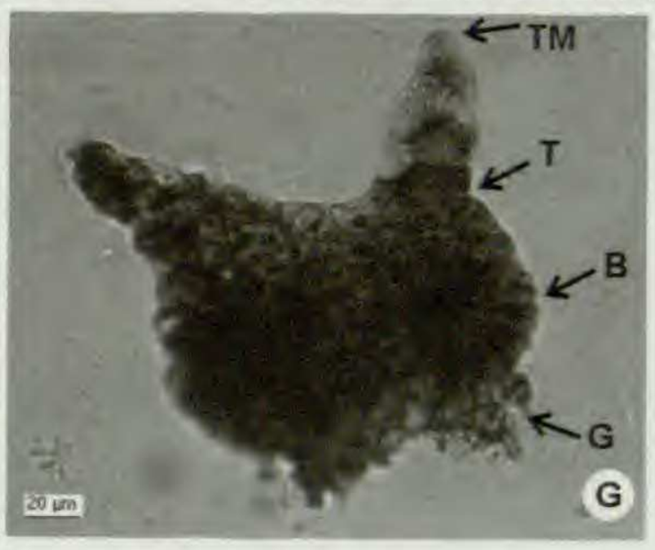


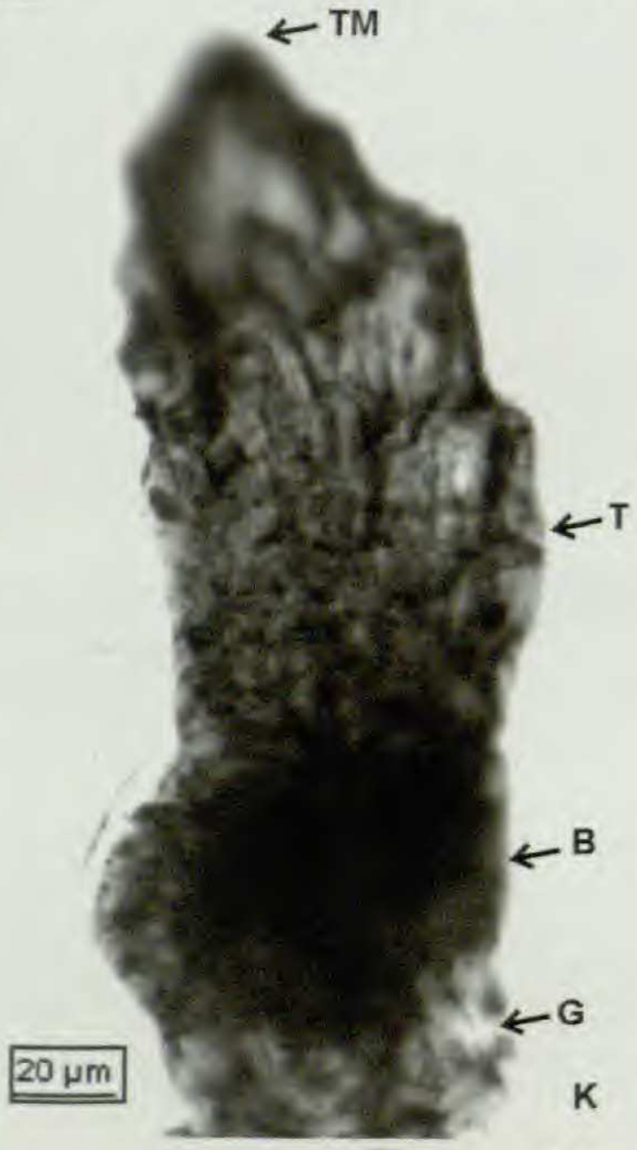
FIG. 1. A–C. Ligules of *S. panchghaniana*. D–G. Leaves of *S. panchghaniana*. A–B. Tongue-like with unlobed and obtuse margin. C. Tongue-like with unlobed and acute margin. D & E. Dorsal and ventral leaves with single ligule, respectively. F & G. Dorsal leaves with double ligule of equal and unequal size, respectively. TM = Tip margin, T = Tip part, B = Basal part, G = Glassopodium, L = Ligule.

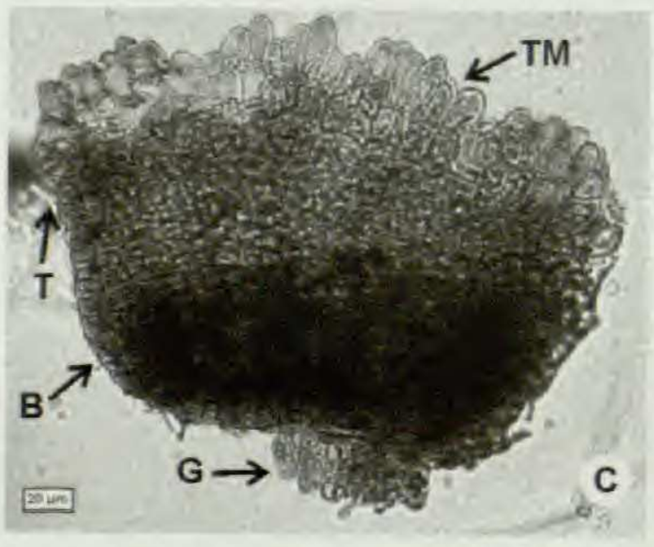


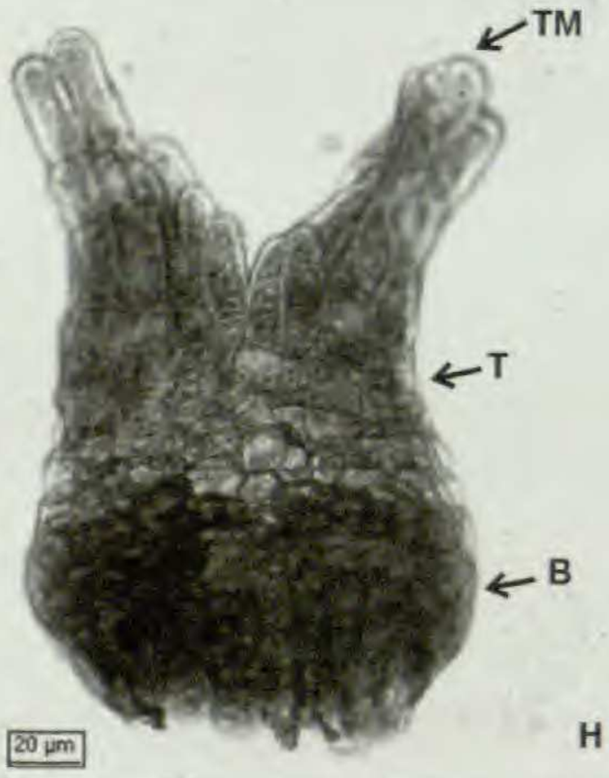


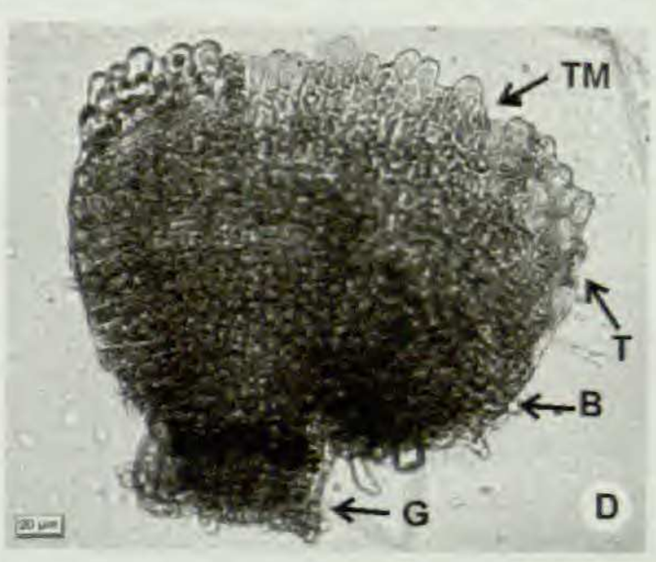


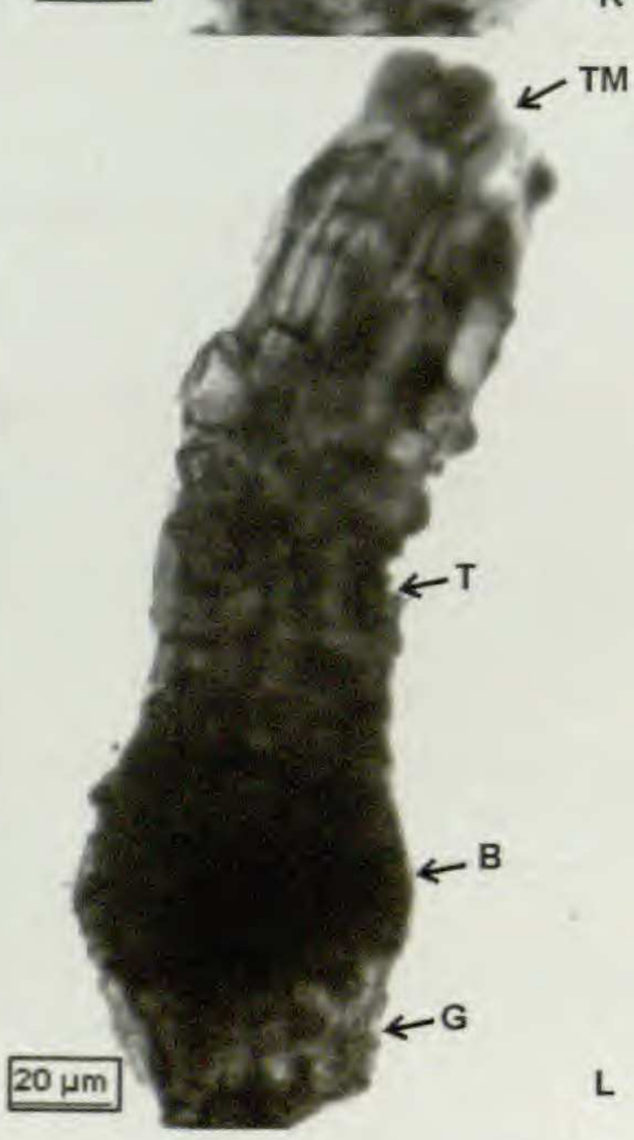


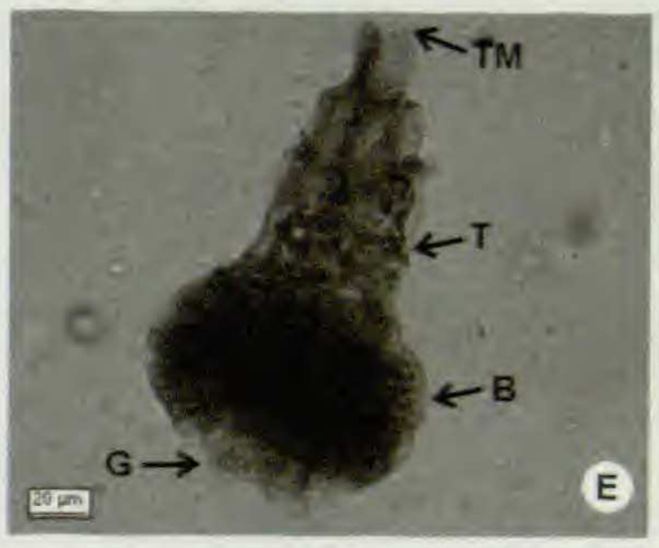












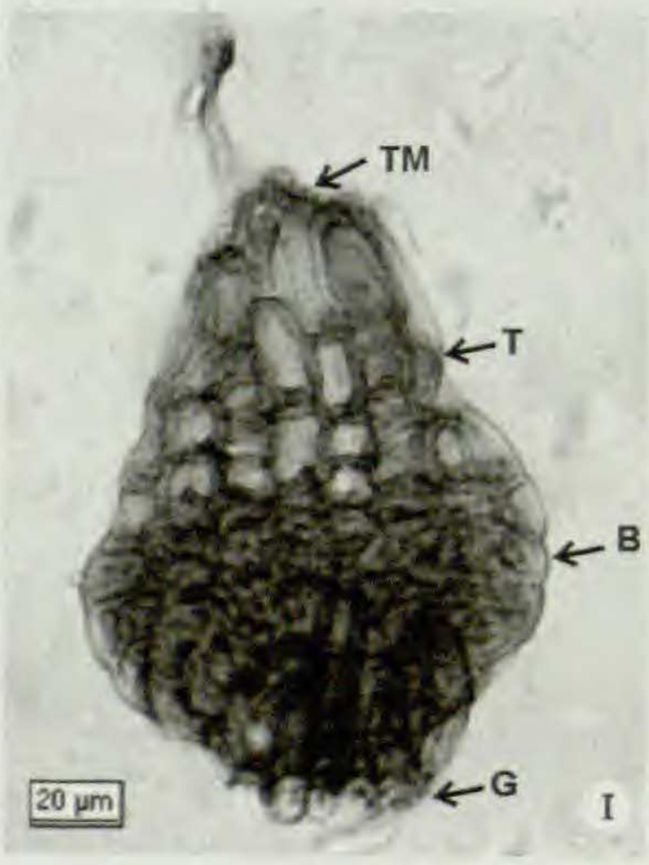


FIG. 2. Ligules. A–D. *S. bryopteris*; E–I. *S. repanda* and J–L. *S. panchghaniana*. A. Cup-shaped with trilobed tip and crenate margin. B–D. Cup-shaped with multilobed tip and crenate margin. E. Tongue-like with unlobed an obtuse margin. F. Heart-shaped with trilobed tip and obtuse margin. G & H. Heart-shaped with bilobed tip and obtuse margin. I. Tongue-like with unlobed and obtuse margin. J. Tongue-like with bilobed tip and acute margin. K–L. Tongue-like with unlobed and obtuse margin. TM = Tip margin, T = Tip part, B = Basal part, G = Glassopodium.

TABLE 1. Showing the differences in size (length and breadth) of ligules of three species of *Selaginella*.

Name of species	Habitat condition	Shape, tip and margin of tip of ligules		Ligules of ventral leaves. Min. (μm)	Max. (μm)	Av. (μm)	S.D.	CV.	Ligules of dorsal leaves. Min. (μm)	Max. (μm)	Av. (μm)	S.D.	CV.
S. bryopteris	Highly dry and open	Cup–shaped or fan-shaped; multi to tri- lobed; crenate.	L.	165	285	216.5	23.2	10.7	165	255	210	22.9	10.9
			B.	150	420	260.9	63.4	24.3	120	457.5	245.1	55.2	22.5
	Dry and open	As above	L.	135	225	189.1	20.3	10.7	105	240	177.5	21.6	12.2
			B.	127.5	225	224.1	55.8	24.9	82.5	300	197.3	45.5	23.1
S. repanda	Semi wet and open	Horn- shaped and tongue-like; tri, bi and unlobed; obtuse.	L.	142.5	285	207.1	33.8	16.3	142.5	270	212.5	28.2	13.3
			B.	67.5	135	103.9	18.3	17.6	67.5	120	93.5	13.2	14.2
S. panchghaniana	Wet and shaded	Only tongue-like; unlobed, rarely bilobed; obtuse to acute.	L.	105	345	214.6	47.2	21.9	105	332.5	220.5	41.5	18.8
			B.	45	97.5	69.2	12.7	18.4	37.5	105	60.1	12.3	20.4

L. = Length, B. = Breadth.

two cases of the presence the two ligules of unequal size in a single sporophyll of *S. martensii* in classroom slides. Webster (1970) successfully developed aberrant leaves from an excised angle shoot meristem with 2–5 tips, as well as mid-ribs and single or double ligules of equal size. The presence of dorsal leaves with more than one tip and ligule in Indian species of *Selaginella* is in agreement with earlier reports. However, these reports are confined either to classroom examination of slides or cultural conditions. We report, for the first time, the occurrence of double ligules in *S. panchghaniana* growing *in situ*. This indicates that some of the species of *Selaginella* have the potential to produce more than one ligule in a single microphyll or sporophyll.

Selaginella bryopteris is characterized by the presence of a radial shoot without aerial roots (rhizophores) and the presence of ligules in a shallow cavity at the leaf base indicates its retention of ancestral traits. Bower (1959), on the basis of radial shoots and absence of aerial roots had regarded that "*S. spinulosa* may be a primitive species of *Selaginella*". This is further supported by Korall *et al.* (1999). They studied generic / sub-generic relationships of Selaginellaceae based *rbcL* gene sequences, and found that the group of *Tetragonostachys* has more extended distribution of rhizophores between branches in many places along the stem and concluded that "the relationships of *Tetragonostachys* indicate that the extended distribution of rhizophores is a derived characteristic". Maslen (1898) and Grierson and Bonamo (1979) also reported that the ligules of *Lepidodendron* and *Leclerquia* respectively, arise from the shallow depression on the adaxial surface of leaves. In our observation the presence of a radial shoot without aerial roots, and ligules in shallow cavity of *S. bryopteris* point out its ancestral nature.

In *S. repanda*, both ventral and dorsal leaves are of normal type with horn-shaped or tongue-like ligules. Fan-shaped and tongue-like ligules have been described by many workers (Harvey Gibson, 1896; Vink, 1953; Horner and Beltz, 1975; and Dahlen, 1988), but no one has described horn-shaped ligules. Ours is the first report of horn-shaped ligules in *S. repanda*.

The plants of *S. bryopteris*, *S. repanda* and *S. panchghaniana* are found growing in different ecological conditions. The ligules of xerophytic species (*S. bryopteris*) are tougher, thin and light colored, generally with more than one lobe of which the tip margins are crenate with many glandular cells (Fig. 2 A–D). The ligules of *S. repanda* are loose, thick, dark colored and tri-, bi-, or unlobed, and tip margins are obtuse with few glandular cells (Fig. 2 E–I). The ligules in the mesophytic species (*S. panchghaniana*) are loose, thick, dark colored, and unlobed. The ligules are occasionally divided and their tips are obtuse to acute, with fewer glandular cells than *S. repanda* (Fig. 2 J–L; Fig. 1 A–C). Our observations on ligules of xerophytic species is similar to those of *S. cauliscens* and *S. rupestris* described by Vink (1953) and *S. lepidophylla* described by Jagels and Garner (1979). These species are xerophytic and the structure of the ligules is almost same in all the species. The ligules of xerophytic species are more prominent and robust than other species. It suggests a structural adaptation due to the stressful conditions and greater water demand (see Table 1). The presence of glandular cells at the margin of

the tips of ligules further supports that the major function of ligules may be secretion.

No specific function of the ligule is known but a number of hypotheses have been proposed to elucidate the function of ligules in *Selaginella*. The hypothesized function of ligules can be grouped into three categories: organ of absorption (Mc Nab, 1887; Kohlenbach and Geier, 1970); secretion of water or mucilage (Hofmeister, 1851; Harvey Gibson, 1896; Maslen, 1898; Foster and Gifford, 1959; Bierhorst, 1971; Bilderback and Slone, 1987; and Bilderback, 1987); production of callose (Jagels and Garner, 1979) or complex carbohydrate (Sigee, 1976). However, Lerbs (1974), Lafont and Lemoigne (1965), Sigee (1974, 1975, 1976) and Horner & Beltz (1975) could not detect any actual exudations of secreted substances in ligule. Sigee (1974) said that ligule is a vestigial organ, having lost its secretory function during the course of evolution. Our observations support the view that the ligule is physiologically active and may be involved in the secretion of water to keep the young leaf moist.

The present study reveals that the morphology of ligules provide an additional taxonomic tool for the delimitation of *Selaginella* species. The varied environmental conditions are associated with the morphometric features of ligules within the plants of same species.

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